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Research Article

PREPARATION OF DIFFERENT CRUDE EXTRACT AND ANTIMICROBIAL STUDIES OF *SPIROGYRA RHIZOPUS*

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ABSTRACT

Objective: The importance of this work is to prepare the crude extracts of *Spirogyra rhizopus* and to study the biological activity of crude extract against four bacterial strains.

Materials and Methods: *Spirogyra* algae collected from Jimma town, and crude extracts were prepared by cold percolation method and sonication method and further analyzed for qualitative phytochemical analysis. The efficacy of crude extracts is tested for bacterial activity by disc-diffusion method.

Results: The maximum zone of inhibition shown by the crude extract is compared to standard and control. Among the four extracts, chloroform extract displayed the promising inhibitory action against four bacterial strains.

Conclusions: The preliminary study concludes that green algae *S. rhizopus* is a potential source of pharmacologically active lead molecules. *In vitro* screening of crude extracts of green algae *S. rhizopus* shows promising activity against bacterial strains and thus suggests its application in drug discovery research.

Keywords: *Spirogyra rhizopus*, Ultrasound, Cold percolation, Phytochemical analysis, Antibacterial activity.

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INTRODUCTION

The medicinal plants are useful for the treatment of human diseases due to the presence of active phytochemical principles [1-6], and they have the advantage for an organism to serve as a chemical defense against predation [7,8]. Algae of marine and terrestrial origins have been significant attraction as source of bioactive molecules [9], and they can produce antibacterial [10], antifungal [11], and antiviral [12] compounds of interest for the drug discovery research program [13,14]. The phytochemical and pharmacological studies on algae prove that they are potential source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes, and cyclic polysulfides [15]. Recent studies on algae showed that it can be useful for the bioremediation of heavy metals, and their ability to accumulate, tolerate high level of metals has been demonstrated [16]. Moreover, algae are one of the most promising sources of renewable biomass [17], for the production of biodiesel [18] and also can able to enrich the soil fertility [19].

Spirogyra species is a freshwater, and filamentous green algae contain high amount of nutritional compositions, which consumed as food in northern Thailand and have pharmacological properties. It contains high amounts of protein, carbohydrate, fat, dietary fiber [20], and mineral substances [21,22]. Amornlerdpison *et al*. reported that the crude extract of *Spirogyra* spp. has potential to inhibit the gastric ulcer formation induced under physical, chemical stress in rats, also displayed anti-inflammatory effect [23], and the recent research on *Spirogyra* spp. shows that the hypolipidemic and hypoglycemic abilities in rats induced by streptozotocin [24]. The *in vitro* studies of crude extract display antioxidant activity [25] and its phytochemical composition revealed the presence of phenolics, tannins, glycosides, and saponins [26].

However, there is no previous report on the preparation of crude extract of *Spirogyra rhizopus* and antibacterial activity studies of different solvent crude extracts. Hence, the focus of the present study is to carry out phytochemical qualitative analysis of crude extracts of *Spirogyra* species and study on antimicrobial activities of different crude extract. These tests were carried out following standard procedures reported in literature.

MATERIALS AND METHODS

Sample collection: Fresh *Spirogyra* spp. sample was collected from Kito around Jimma. The collected species were identified as *S. rhizopus* by the plant taxonomist in the Department of Life Sciences, Jimma University, Ethiopia.

Ultrasound for extraction is generated with the help of ultrasonic instrument. The specifications, operating parameters, and the details are as follows. Designed and made by China, operating frequency: 36 ± 3 kHz; rated output power: 700W; and tank size: 240 mm × 135 mm × 100 mm.

Preparation of crude extracts

The fresh *S. rhizopus* was washed under tap water and distilled water to remove associated debris, dried under shade, and further grounded into fine powder using electric blender. The method of preparation of crude extract as described below.

Sequential solvent extraction of *S. rhizopus*

The procedure of preparing crude extracts of the *S.rhizopus* was conducted in accordance with reported method [27]. Extraction was carried out at room temperature under normal condition by maceration technique also called as cold percolation method. The dry powder was weighed accurately and subjected to extraction in an orbit shaker apparatus with solvent in the sequential order of increasing polarity as petroleum ether, chloroform, acetone, and methanol successively for 72 h with constant continuous shaking. Before carry on the extraction with the next solvent, residue was air dried to remove the adhering solvent, and the same procedure was followed.

Sequential extraction by petroleum ether, chloroform, acetone, and methanol

Dried and powdered material of *S. rhizopus* was subjected to sequential solvent extraction with petroleum ether, chloroform, acetone, and methanol using maceration technique, and antibacterial studies were carried out using *in vitro* method. About 300 g dry powder of *S. rhizopus* was soaked in 1000 ml of petroleum ether in airtight conical flask for 72 h on an orbit shaker and filtered, and filtrates were collected into airtight bottles. The same procedure was followed with fresh petroleum ether; the filtrates were pooled together; the solvent was distilled under vacuum by rotary evaporator; and the resulting crude extract was stored in deep freezer (−20°C) until further study. The residue of the algal powder was dried and used for further sequential, gradient extractions with an equal volume of chloroform, acetone, and methanol; the same procedure was followed; and different crude extracts were prepared, the results are displayed in Table 1.

Twenty gram of dry, powdered *S. rhizopus* sample was taken and sequentially extracted with different solvents (petroleum ether, chloroform, acetone, and methanol) using ultrasonic instrument (45 kHz, 210 kHz, and 1 MHz). The weight of the crude extracts and their percent yields by sonication method are given in Table 2.

Comparison of the yield of crude extract by maceration and sonication method

The influence of ultrasound radiation on the yield of extraction with various solvents was studied, and influence on yield is negligible. However, in microwave method, violent eruption of solvent was observed even under low intensity of microwave irradiation.

Phytochemical analysis

The extracts were subjected to phytochemical tests to confirm the presence of biomolecules using standard qualitative procedures as described in literature [28], and the results are presented in Table 3.

Antibacterial activity studies

Procedure of antibacterial test of algae extracts

For testing the antibacterial activity of different solvent crude extracts, the following bacterial strains *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella typhimurium* were obtained from the Microbiology Department, Addis Ababa University, Addis Ababa, Ethiopia. The bacterial culture of *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *S. aureus* was pre-cultured in nutrient broth. The media were incubated overnight at 35°C in rotary shaker and it was centrifuged at 10,000 rpm for about 5 min. The pellet was suspended in distilled water, and the turbidity was compared with 0.5 McFarland standards to ensure the suspension approximately 10^8 cfu/ml. The activity was tested using disc-diffusion method [29], prior to that bacterial suspension was spread evenly onto the Muller Hilton Agar plates by using sterile cotton swab. The solvent crude extracts of algae samples were tested in a dose level of 100 mg/ml, and the medium in the plates was allowed to set at room temperature for 10-20 min at room temperature for the diffusion of the extract into the agar and finally, incubated at 35°C for 24 h for bacterial growth. After incubation, the antibacterial activity was indicated by a clear zone of inhibition and measured with the help of a ruler and the results are displayed in Table 4. Dimethyl sulfoxide (DMSO) will be used as negative control against all the bacterial pathogens, and gentamycin was used as positive control for *S. aureus, E. coli, P. aeruginosa,* and *S. typhimurium*.

Evaluation of antibacterial effects with agar disc diffusion method

Whatman No.3 filter paper (sterilized) disks (6 mm in diameter) was placed onto the surface of inoculated Petri dishes, and the *S. rhizopus* crude extracts (petroleum ether, chloroform, acetone, and methanol) were dissolved in DMSO and were placed separately on the inoculated Petri dishes. In brief, the extract disc contained 100 µl of extract, positive control disc contained gentamycin (10 µg/disc), and the negative control disc contained 100 µl of DMSO. The Petri dishes were then allowed for 10-20 min at room temperature for the diffusion of the extract into the agar and finally, incubated at 30°C for 24 h for bacterial growth. The antibacterial activity was indicated by a clear zone of inhibition and measured with the help of a ruler, and the results are displayed in Table 4 and zone of inhibition shown in Fig. 1. diameters of inhibitory zones induced by the extracts were interpreted as follows: resistant (12 mm or less), intermediate (13-15 mm), and susceptible (16 mm or above), followed the standard. MIC values were determined as the lowest concentration that completely inhibited the growth of bacteria after 24 h of incubation at 29°C for 24 h.

Table 1: Amounts of crude extracts obtained by cold percolation method

Solvents	Mass of crude extract (g)	%yield
Petroleum ether	4.227	1.409
Chloroform	10.405	3.468
Acetone	5.748	1.916
Methanol	5.437	1.812

Table 2: Amounts of crude extracts obtained by ultrasound assisted extraction method

Solvents	Mass of crude extract (g)	%yield
Petroleum ether	0.380	1.9
Chloroform	0.7668	3.83
Acetone	0.446	2.23
Methanol	0.428	2.14

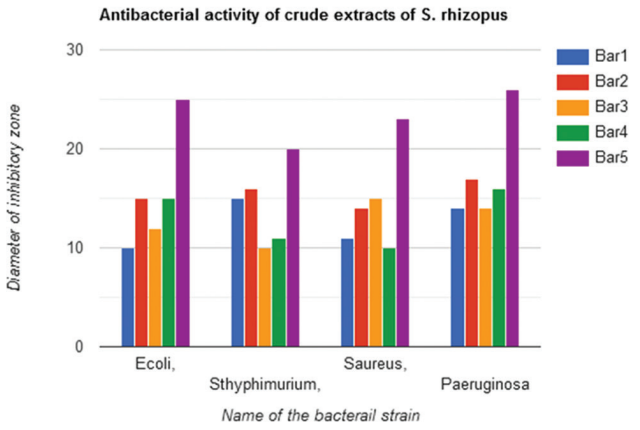

Table 3: Phytochemical screening of *Spirogyra* spp. extracts of different solvents

Phytochemical	Petroleum ether	Chloroform	Acetone	Methanol
Alkaloid	-	-	-	-
Terpene	+	+	+	-
Tannin	-	+	+	+
Saponin	-	+	+	+
Flavonoid	-	-	-	+
Anthraquinone	-	-	+	-

- sign confirms the absence of particular phytochemical and + sign shows that presence of particular phytochemical in the crude extract

Table 4: Antibacterial activity of *Spirogyra rhizopus* crude extracts of different solvents

Solvent	*Escherichia coli*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Petroleum ether	10	15	11	14
Chloroform	15	16	14	17
Acetone	12	10	15	14
Methanol	15	11	10	16
Gentamicin*	25	20	23	26
DMSO*,#	–	–	–	–

Values are in mm; *(+) ve control; *,#(−) ve control, No inhibition zone. DMSO: Dimethyl sulfoxide

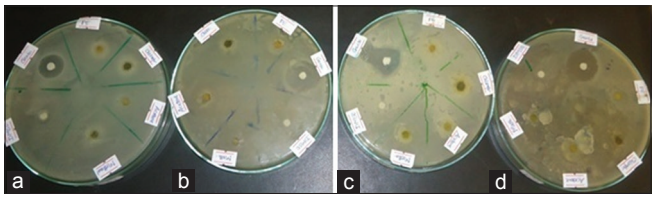

Fig. 1: (a-d) Antibacterial activity of *Spirogyra rhizopus* crude extracts of different solvents. (a) *Salmonella thyphiurium*, (b) *Pseudomonas aeruginosa*, (c) *Staphylococcus aureus*, (d) *Escherichia coli*

X-axis: Bacterial strain; Y-axis: Zone of inhibition (mm) and each bar is crude extract; Name of the crude extracts: Bar 1: Petroleum ether; Bar 2: Chloroform; Bar 3: Acetone; Bar 4: Methanol; Bar 5: Gentamicin

RESULTS AND DISCUSSION

Antibacterial activity studies of *S. rhizopus*

Evaluation of antibacterial activity for different solvent crude extracts was carried out by standard procedure. The petroleum ether, chloroform, acetone, and methanol extracts of *S. rhizopus* were tested against four bacterial pathogens *S. typhimurium, P. aeruginosa, S. aureus, and E. coli*, the results are presented in Table 3, and the degree of activity was varied with reference to different solvent extracts of *S. rhizopus*; from table, chloroform extracts were found to show the maximum zone of inhibition against *S. typhimurium* and *P. aeruginosa*. The acetone extract showed maximum zone of inhibition against *P. aeruginosa* and *S. aureus*, followed by *E. coli*. The petroleum ether extract showed maximum zone of inhibition against *S. typhimurium*, followed by *P. aeruginosa*. The methanol extract showed maximum zone of inhibition against *P. aeruginosa* followed by *E. coli*.

CONCLUSIONS

Extensive efforts for the identification of bioactive compounds derived from natural resources have been made worldwide, to develop safe, nontoxic, and efficient antimicrobial agents of valuable practice in pharmacology. The present study concludes that green algae *S. rhizopus* is a rich and varied source of pharmacologically active natural products. *In vitro* screening of organic solvent extracts of green algae *S. rhizopus* shows promising activity against bacterial strains. Among all the organic extracts chloroform crude extract showed excellent effect of bacterial inhibition. Further column chromatography isolation of lead molecules, characterization, and bioactivity studies will publish in the due course of time.

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AUTHORS' CONTRIBUTIONS

Collection of *S. rhizopus* (algae) material from Jimma town, park, lake, and ponds: Daniel, venkatesan jayakumar, Jimma University.

Identification and processing of the collected Spirogyra algae: Nair, Taxonomist, Department of Life Science, Jimma University.

Design and Preparation of solvent crude extracts, phytochemical analysis: Girma, Venkatesan jayakumar, Daniel, Jimma University.

Collection of bacterial strain, antibacterial assay and statistical analysis: Birtukan, Department of Microbiology, University of Addis Ababa, Daniel, Venkatesan jayakumar, Jimma University.

Writing original draft, review and editing: Girma, Venkatesan jayakumar, Jimma University.

CONFLICTS OF INTEREST

No conflict of interest and the authors authenticate that there are no known conflicts of interest affiliated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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